# Impact of chemical elicitor applications on greenhouse tomato plants and population growth of the green peach aphid, *Myzus persicae*

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## Abstract

Recent advances in the understanding of plant signaling pathways have opened the way for using elicitor-induced plant resistance as a tactic for protecting plants against arthropod pests. Four common elicitors of induced responses in tomato, *Lycopersicon esculentum* Mill. (Solanaceae), were evaluated with regard to phytotoxicity, induction of plant defensive proteins, and effects on population growth and fecundity of a common pest, the green peach aphid, *Myzus persicae* (Sulzer) (Homoptera: Aphididae). Ethephon and methyl jasmonate (MJ) treatments caused varying degrees of phytotoxicity. Ethephon caused pronounced changes in plant growth form and severe, dose-dependent negative impacts on plant growth and flowering. Effects with MJ were milder, but still caused temporary inhibition of development, leading to smaller plants and delayed flowering. The commercial elicitors benzothiadiazole (BTH) and harpin did not cause detectable phytotoxicity. The highest doses of ethephon and MJ significantly increased leaf peroxidase (POD) levels but only MJ treatments significantly increased polyphenol oxidase (PPO) levels. BTH and harpin had no detectable effects on POD and PPO. Populations of green peach aphids grew significantly more slowly on plants treated with BTH or MJ than on control plants or plants treated with harpin or ethephon. Slowed aphid population growth on BTH-treated plants was due to significant reductions in aphid fecundity, although this was independent of changes in time to onset of reproduction or time to death. Aphid fecundity was also reduced on MJ-treated plants relative to controls, but this difference was not statistically significant, suggesting that other mechanisms are involved in slowing aphid population growth on MJ-treated plants. Growth of aphid populations on plants treated with a MJ–BTH mixture was reduced almost as much as with treatments of MJ alone, suggesting that antagonism between JA-dependant and SA-dependent plant signaling pathways is only mild with regard to induced defenses against aphids.

## Introduction

Induced resistance to herbivory or plant pathogens has been documented in over 100 plant systems (Karban & Baldwin, 1997; Heil & Bostock, 2002) including many cultivated crops (Gorlach et al., 1996; Korth & Dixon, 1997; Stout et al., 1998a; Kahl et al., 2000; Omer et al., 2001; Engelberth et al., 2004). Better understanding of the signaling pathways in plants that control induced responses has led to the discovery of natural and synthetic compounds, called elicitors, that induce responses in plants similar to those triggered by natural herbivory or pathogen infection (Karban & Kuc, 1999). At least six signal pathways have been implicated in plant responses to pathogens and herbivores (Walling, 2000). Four of the pathways, (1) the reactive oxygen species (ROS)/ nitric oxide (NO) pathway, (2) the salicylic acid (SA) pathway, (3) the jasmonic acid (JA)/ethylene sequential pathway, and (4) the JA/ethylene concomitant pathway are frequently associated with responses to pathogens. The other two pathways, (1) the JA-dependent wound pathway, and (2) the JA-independent wound pathway, are primarily associated with herbivore feeding. Many workers have

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proposed the use of elicitors of induced plant resistance as a means of controlling arthropod pests and diseases in agriculture (Karban & Baldwin, 1997; Inbar et al., 1998; Thaler et al., 1999). Most elicitors have low toxicity and may constitute alternatives to toxic chemical pesticides, offering improvements in farm worker safety, reduced environmental impact, and potential compatibility with biological control by natural enemies.

Four common elicitors of induced plant responses are methyl jasmonate (MJ), the SA mimic benzothiadiazole (BTH), ethephon, and the bacterial protein harpin. JA or its methyl ester MJ has been shown to induce resistance to arthropods in many cultivated crops (Avdiushko et al., 1997; Havill & Raffa, 1999; Omer et al., 2001; Black et al., 2003). In tomato, applications of exogenous JA or MJ activate the JA-dependent wound pathway, causing induction of the same defensive proteins, polyphenol oxidase (PPO), peroxidase (POD), proteinase inhibitors, and lipoxygenase, induced by herbivore feeding (Stout et al., 1998b; Cipollini & Redman, 1999; Thaler, 1999). These proteins are associated with increased plant resistance to herbivores by reducing herbivore preference, performance, and reproduction (Broadway & Duffey, 1986; Felton et al., 1989; Duffey & Felton, 1991; Stout & Duffey, 1996; Thaler et al., 1996). BTH brings about responses normally associated with plant pathogens (Lamb & Dixon, 1997; van Loon, 1997), conferring resistance throughout the plant to a broad range of fungal, bacterial, and viral pathogens in a phenomenon known as systemic acquired resistance (SAR) (Heil & Bostock, 2002). However, a recent study has suggested that these responses may also confer resistance to aphids (Cooper et al., 2004). Ethephon is metabolized in plants to release ethylene, which activates the ethylene/JA-dependent pathogen response pathway causing expression of pathogenesis-related (PR) proteins (Hong et al., 2000; Lee et al., 2001). Harpin is a protein from the bacterium *Erwinia amylovora* that causes fire blight disease in apples. Harpin activates the SA- and ethylene/JA concomitant pathways, inducing pathogen resistance (Wei et al., 1992).

Elicitors of induced plant responses have potential for use in managing common pests and diseases of agricultural crops, but studies are needed to evaluate their effectiveness against pests and impacts on plant growth. In greenhouse studies, using tomato as a model system, we evaluated four elicitors, MJ, BTH, ethephon, and harpin, to identify concentrations for each elicitor that maximized induction of plant defensive responses, as indicated by induction of the defensive proteins PPO and POD. Subsequent experiments evaluated the impact of these elicitor treatments on population growth and fecundity of a common tomato pest, the green peach aphid, *Myzus persicae* (Sulzer) (Homoptera: Aphididae). Effects of elicitor applications on growth and flowering of tomato plants were also assessed.

## Methods

### Plants

Tomato plants (*Lycopersicon esculentum* cv Trust) (DeRuiter Seeds Inc., Lakewood, CO, USA) were grown in 10-cm plastic pots in sterile soil mix (peat-perlite-vermiculite, 55-20-25; Penn State Seed, Dallas, PA, USA). Plants were grown hydroponically in a greenhouse under natural lighting with day and night temperatures varying between 21 °C and 33 °C. Plants were irrigated daily with a fertilizer solution (N-P-K, 4-18-38; Chem-Gro Tomato Formula, Hydro-Gardens Inc., Colorado Springs, CO, USA) containing supplemental magnesium sulphate and calcium nitrate.

### Insects

A colony of green peach aphids, *M. persicae*, was maintained on tomato plants in isolation cages in the greenhouse. The colony was established 3 months prior to the onset of experiments, to ensure that aphids were suitably adapted to the chemistry of tomato plants.

### Elicitors

Elicitors consisted of BTH (Actigard®; Syngenta, Greensboro, NC, USA), harpin (Messenger®; Eden Biosciences, Bothell, WA, USA), ethephon (2-chloroethylphosphonic acid; Sigma, St. Louis, MO, USA), and MJ (Bedoukian Research, Danbury, CT, USA).

*Elicitor-induced phytotoxicity and effects on plant growth.* Five leaf-stage tomato plants were sprayed with high, medium, or low doses of one of four elicitors, BTH, harpin, ethephon, or MJ. Solutions of BTH, harpin, or ethephon were dissolved in distilled water. High, medium, and low doses corresponded to: BTH – twice label (0.26 mM), label (0.13 mM), or half label (0.07 mM) rates, respectively; harpin – twice label [0.012% (wt/vol)], label [0.006% (wt/vol)], or half label [0.003% (wt/vol)] rates, respectively; ethephon – 10 mM, 5 mM, or 1 mM solutions, respectively. For MJ treatments, high, medium, and low doses consisted of 10 mM, 7.5 mM, or 5 mM solutions of MJ, respectively, dissolved in 0.8% ethanol and distilled water. Control solutions consisted of distilled water alone or 0.8% ethanol and water.

Plants were randomly assigned to different treatments and were removed from the greenhouse prior to application of solutions. Solutions were applied using separate hand atomizers for each elicitor, starting with the low dose and working up to the highest dose. Plants were sprayed until leaves were saturated and then left for 40 min to dry off before being returned to the greenhouse. Treated plants were arranged on three benches according to a randomized complete block design with three plants per treatment per bench. Benches were treated as experimental blocks. Plants

were subsequently monitored for elicitor-induced phyto-toxicity. At 72 h post-treatment, terminal leaflets of the fifth leaf of each plant were harvested and frozen at −80 °C for subsequent enzyme analysis. At 20 days post-treatment (DPT), height, number of flower buds, and number of open flowers were recorded for each plant.

*Effects of elicitor treatments on leaf enzymes.* Leaf samples were thawed and analyzed for the enzymes PPO and POD using methods based on Thaler et al. (1996), with the only major change being the use of 3 mM chlorogenic acid in place of 3 mM caffeic acid as the enzyme substrate in PPO assays. Briefly, weighed leaf samples were homogenized in an ice-cold mixture of 0.6 ml 10% (v/v) Triton X-100 in 0.1 M dipotassium hydrogen phosphate buffer (pH 7) and 2 ml of 7% (wt/vol) polyvinylpolypyrrolidone in 0.1 M dipotassium hydrogen phosphate buffer (pH 7). Following homogenization, samples were centrifuged at 7500 *g* at 4 °C for 15 min. Immediately following centrifugation, supernatants were transferred into Eppendorf tubes and placed on ice for immediate use in spectrophotometric assays for PPO and POD. For PPO assays, 200 µl volumes of 3 mM chlorogenic acid in 0.1 M dipotassium hydrogen phosphate buffer (pH 7) were added using a multichannel pipette to 5 µl aliquots of leaf extract supernatants in 96-well plates, and the plate immediately read in a SpectraMax 190 Microplate Reader (Molecular Devices, Sunnyvale, CA, USA) to determine increases in optical density at 470 nm ($OD_{470}$). For POD assays, 200 µl volumes of 3 mM guaiacol and 1 mM hydrogen peroxide in 0.1 M dipotassium hydrogen phosphate buffer (pH 7) were added using a multichannel pipette to 5 µl aliquots of leaf extract supernatants in 96-well plates, and the plate immediately read for increases in $OD_{470}$. Changes in optical density were measured as kinetic reactions, with estimates of rates of change of optical density averaged over six wells per leaf extract. Enzyme activities were expressed as change in $OD_{470}$ per min per gram of leaf tissue.

*Aphid population growth studies.* Five leaf-stage tomato plants were infested with green peach aphids by manually transferring 10 aphids onto the fourth leaf of each plant. Infested plants were then moved into large isolation cages, with three plants per cage and six cages arranged on each of three benches. After a 2-day acclimation period, differences in aphid numbers arising from reproduction were equalized by reducing aphid numbers back down to 10 per infested leaf, followed by enclosure of each infested leaf in a plastic bag, taking care not to disturb the aphids. Immediately thereafter, cages on each bench were randomly assigned to one of six treatments: (1) 0.13 mM BTH in water, (2) 0.006% (wt/vol) harpin in water, (3) 4 mM ethephon in water, (4) 7.5 mM MJ in 0.8% ethanol and water, (5) 0.8% ethanol

and water control, and (6) water-only control. These elicitor concentrations were chosen using maximal induction of POD and PPO as the selection criterion. Plants within each cage were sprayed with the appropriate treatment as described previously. Large sheets of cardboard were used to prevent spray drift onto plants in adjacent cages. Immediately following application of treatments, screening bags were carefully removed from infested leaves to avoid dislodging any aphids. Plants were examined at 1, 3, 5, 7, 10, 13, and 17 days post-infestation and the number of aphids on each plant counted.

Trials were repeated on a second occasion, but slightly different methodology was used to save time. Four leaf tomato seedlings were manually infested by transferring 10 first-stage green peach aphid nymphs onto the third leaf of each plant. Infested plants were then moved into isolation cages and left for 24 h. At the end of this time, aphid-infested leaves were carefully enclosed in plastic bags. Cages were randomly assigned to receive one of six treatments: (1) 0.13 mM BTH, (2) 0.006% (wt/vol) harpin, (3) 4 mM ethephon, (4) 7.5 mM MJ, (5) mixture of 0.13 mM BTH and 7.5 mM MJ, or (6) 0.8% ethanol and water control. All treatments were made up in 0.8% ethanol and water. Plants within each cage were sprayed with the appropriate treatment as described previously, and screening bags were subsequently removed. Plants were examined at 4, 8, 11, 14, and 18 days post-infestation and the number of aphids on each plant counted.

*Effects of elicitors on aphid fecundity.* Four leaf-stage plants were randomly assigned to one of three treatments: (1) 0.13 mM BTH in 0.8% ethanol and water, (2) 7.5 mM MJ in 0.8% ethanol and water, or (3) 0.8% ethanol in water control. Plants receiving different treatments were moved to separate sections of the greenhouse and sprayed until saturated, as described previously. Plants were left for several hours to dry. Single plants from each of the three treatments were selected at random and arranged in triplets, with five such triplets of plants on each of three benches, for a total of 15 plants per treatment. Each plant was infested with a single, 1-day-old green peach aphid nymph that was confined on the terminal leaflet of the second leaf by means of a modified Petri dish. Each 10-cm Petri dish had a slot cut in the side of the base for the leaf stem and holes in the top and base of the dish, which had been covered with fine mesh screening (thrips-proof netting, Hydro-Gardens Inc., Colorado Springs, CO, USA) to allow ventilation. Cotton balls were wrapped around the leaf stem where it entered the Petri dish to prevent aphid escape and cages were supported by means of twist ties and canes. Petri dish cages were examined daily and new offspring nymphs produced by the founder aphid since the previous day were counted and removed. Monitoring continued until all the

founder aphids had died, so that total lifetime reproduction was measured for each founder aphid.

### Data analysis

Data were analyzed using general linear models for analysis of variance (ANOVA). Data were checked for conformity to ANOVA's underlying assumptions of normality of error and homogeneity of variance by examining plots of residuals and predicted values and when necessary data were transformed to fix departures from these assumptions. Initially, full models incorporating block (bench), main effect, and interaction terms were fitted. Nonsignificant interaction terms were subsequently removed, and models fitted that included only block and main effects. Plant heights, flowering data, leaf enzyme activities, and aphid fecundity data were analyzed using the univariate ANOVA procedure of SPSS statistical software (SPSS Inc., Chicago, IL, USA) with post-hoc comparison of means using Tukey's means separation test. Aphid population growth data and aphid fecundity data were square-root transformed to stabilize the variance and normalize error. Aphid population growth data were analyzed using the repeated measures ANOVA procedure of SPSS, with post-hoc comparison of means performed using Dunnett's test.

## Results

### Phytotoxic effects

Applications of certain elicitors caused phytotoxicity (Figure 1). Most notably, all concentrations of ethephon caused disruptions of plant growth form that appeared within hours following treatment application. By three DPT, ethephon-treated plants exhibited unusual spiral down-turning of leaves (Figure 1A) and, in the case of the intermediate and high ethephon doses, these effects persisted throughout the duration of the study (Figure 1D). Plants treated with MJ showed low levels of dose-dependent leaf burning, evident as pale-colored necrotic lesions on treated leaves (Figure 1B). Foliage on MJ-treated plants was also a noticeably lighter shade of green by four DPT than foliage of plants treated with other elicitors or control solutions (Figure 1C). Applications of BTH and harpin caused no visible phytotoxicity.

### Effect of elicitors on plant growth and flowering

Treatment with ethephon and MJ caused changes in plant growth and flowering. Plants treated with all concentrations of ethephon exhibited significant changes in growth form, and although those treated with the lowest dose partially recovered, those treated with the intermediate and upper doses exhibited permanent stunting of growth (Table 1). Plants receiving MJ treatments exhibited slight inhibition of growth and delayed development (Figure 1D), but otherwise appeared unaffected, and went on to flower and produce fruit normally. ANOVA on plant heights measured at 20 DPT revealed a significant elicitor*dose interaction ($F_{6,94} = 33.16$, P<0.001), indicating that both elicitor and dose had significant effects on plant height, and that some elicitors had different effects on plant height depending on dose. Mean heights of plants treated with BTH or harpin did not vary across doses, and were not significantly different from plants treated with water-only or water–ethanol controls (Table 1). Plants treated with MJ were significantly shorter in height by 10–15 cm on average than controls, but heights

| Treatment[1] | Mean plant height (m) | Mean total number of flowers[2] | Mean number open flowers |
|---|---|---|---|
| BTH H | 0.65 ± 0.01a | 12.3 ± 0.8ab | 4.9 ± 0.2a |
| BTH M | 0.62 ± 0.02ab | 10.3 ± 0.5abcd | 3.7 ± 0.2ab |
| BTH L | 0.64 ± 0.01a | 11.8 ± 1.1abc | 4.8 ± 0.6a |
| Harpin H | 0.64 ± 0.01a | 10.7 ± 0.4abc | 4.4 ± 0.5ab |
| Harpin M | 0.62 ± 0.01ab | 10.4 ± 0.6abcd | 3.6 ± 0.4ab |
| Harpin L | 0.62 ± 0.01ab | 10.7 ± 0.6abc | 4.3 ± 0.2ab |
| Ethephon H | 0.25 ± 0.01f | 0.0 ± 0.0e | 0.0 ± 0.0c |
| Ethephon M | 0.33 ± 0.01e | 0.0 ± 0.0e | 0.0 ± 0.0c |
| Ethephon L | 0.50 ± 0.01d | 13.0 ± 1.0a | 3.2 ± 0.3b |
| MJ H | 0.53 ± 0.01cd | 7.6 ± 0.9d | 0.7 ± 0.3c |
| MJ M | 0.54 ± 0.01cd | 9.4 ± 0.8bcd | 0.6 ± 0.3c |
| MJ L | 0.57 ± 0.01bc | 9.1 ± 0.4cd | 0.2 ± 0.2c |
| Water + ethanol | 0.64 ± 0.01a | 12.2 ± 0.3ab | 3.9 ± 0.2ab |
| Water | 0.63 ± 0.01a | 11.4 ± 0.5abc | 4.1 ± 0.3ab |

**Table 1** Effect of elicitors on growth and flowering of *Lycopersicon esculentum* plants at 20 days post-treatment

[1]High (H), medium (M), and low (L) elicitor doses. Means and SEMs based on nine plants per treatment. Means within the same column followed by different letters are significantly different by Tukey's test (P<0.05).

[2]Mean total number of flowers (buds + flowers).

**Figure 1** Phytotoxic effects on *Lycopersicon esculentum*. (A) Ethephon-treated (10 mM) plant at 3 days post-treatment (DPT) showing changes in growth form due to down-turning of leaves, (B) methyl jasmonate (MJ)-treated (10 mM) plant at 3 DPT, showing leaf spotting/ burning, (C) healthy control plant at 3 DPT, (D) typical size differential at 21 DPT between (left to right) ethephon- (10 mM), MJ- (10 mM) and control-treated plants.

did not differ significantly across MJ doses. Ethephon treatments caused significant dose-dependent reductions in plant height. Plants treated with the intermediate and highest doses of ethephon exhibited severe stunting of growth, and by 21 DPT, were on average only half the height of control plants. Block did not explain a significant amount of the variation seen in height ($F_{2,94} = 2.53$, P = 0.085).

Some elicitor treatments had significant effects on plant flowering (Table 1). ANOVA on total flower numbers (flower buds and open flowers) measured at 20 DPT revealed a significant elicitor*dose interaction ($F_{6,94} = 33.04$, P<0.001), indicating that both elicitor and dose had significant effects on total flower number, and that some elicitors had different effects on total flower number depending on dose. Total flower numbers on plants treated with BTH or harpin did not differ significantly across doses and were not significantly different from flower numbers on plants treated with control solutions (Table 1). Plants treated with MJ did not differ significantly in flower number across doses, but MJ-treated plants did bear fewer flowers than plants treated with water-only or water–ethanol controls. Ethephon had significant dose-dependent effects on flowering, with no flowers present on plants treated with the intermediate and highest doses. Significant differences in total flower numbers were present between blocks ($F_{2,94} = $

4.91, P = 0.009). Analysis of numbers of open flowers present at 20 DPT also revealed a significant elicitor*dose interaction ($F_{6,94} = 10.03$, P<0.001) (Table 1). Patterns were similar to those seen for measurements of total flower numbers. Mean numbers of open flowers did not vary significantly across doses for BTH or harpin, and were not significantly different to mean numbers of open flowers observed on control-treated plants. For MJ-treated plants, numbers of open flowers did not differ significantly across doses, but were significantly lower than flower numbers observed on BTH-, harpin-, or control-treated plants. Ethephon-treated plants exhibited significant dose-dependent effects on numbers of open flowers, due to the absence of flowers on plants treated with intermediate and high doses. Significant differences in numbers of open flowers were present among blocks ($F_{2,94} = 4.92$, P = 0.009).

### Effects of elicitor treatments on leaf enzymes

Elicitor treatment ($F_{3,100} = 195.83$, P<0.001) had a significant effect on PPO activity, but dose did not ($F_{2,100} = 0.80$, P = 0.451). PPO activities in leaves from plants treated with BTH, harpin, or ethephon were not significantly different from those seen in leaves from plants treated with either of the control solutions (Figure 2A). However, PPO activities were significantly higher by approximately threefold
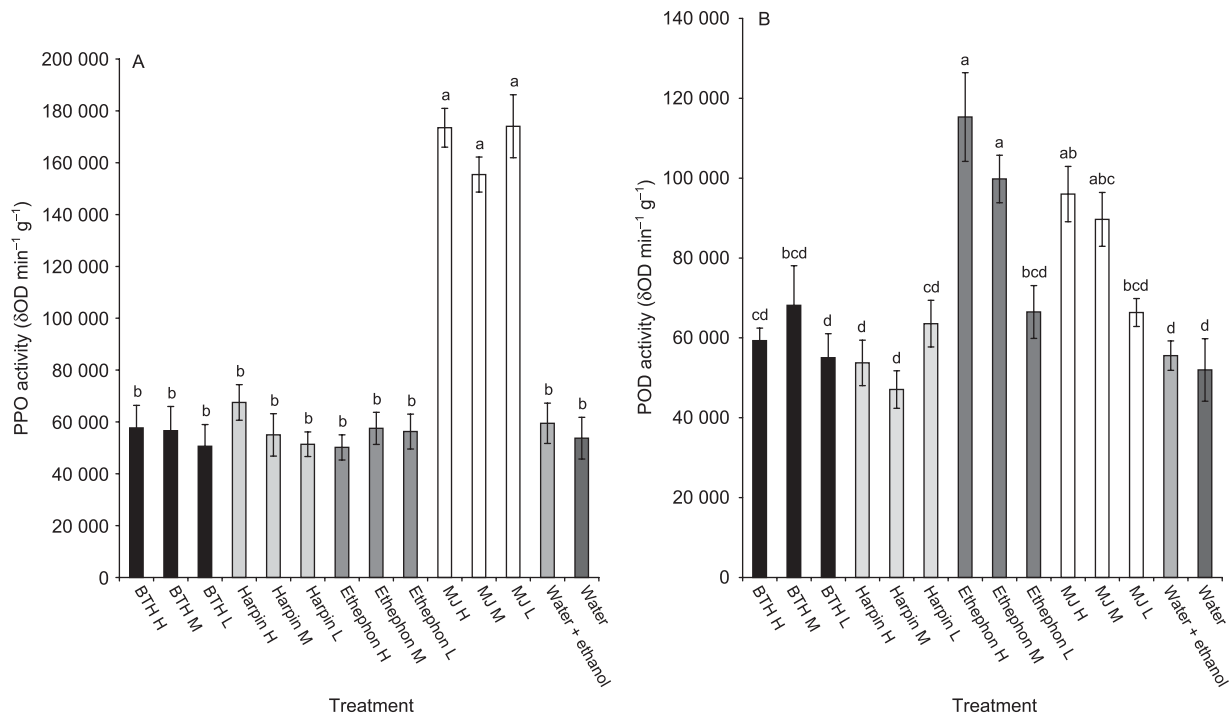


**Figure 2** Histograms showing oxidative enzyme activities in *Lycopersicon esculentum* leaves harvested 72 h post-treatment for high (H), medium (M), and low (L) doses of each elicitor. (A) Total polyphenol oxidase (PPO) and (B) total peroxidase (POD). Means based on leaves from nine plants per treatment. Bars show SEM. Bars with different letters are significantly different by Tukey's test (P<0.05).

in leaves from plants treated with MJ solutions, compared to activities in leaves from plants treated with either of the control solutions. Significant differences in PPO activity were present among blocks ($F_{2,100} = 14.22$, P<0.001).

Both elicitor and dose had significant effects on POD activity, and the presence of a significant elicitor*dose interaction ($F_{6,94} = 4.92$, P<0.001) indicated that the effect of some elicitors on POD activity varied across doses. POD activities in plants treated with BTH and harpin were not significantly different across doses, and did not differ significantly from POD activities in plants treated with water-only or water–ethanol controls (Figure 2B). POD activities were elevated in leaves from plants treated with the intermediate and highest doses of MJ, but these were not statistically different from the mean POD activity observed at the lowest MJ dose. Highest POD activities were seen in leaves from plants treated with the intermediate and high doses of ethephon, which differed significantly from mean POD activity at the lowest ethephon dose, and also from

POD activities seen in BTH, harpin, and control-treated plants. Significant differences in POD activities were present among blocks ($F_{2,94} = 4.12$, P = 0.019).

### Aphid population growth studies

Aphid population growth data were square-root transformed prior to analysis. Repeated measures analysis of variance showed that elicitor treatment of host plants had a significant effect on aphid population growth in both the first ($F_{5,10} = 4.0$, P = 0.030) and the second ($F_{5,10} = 8.0$, P = 0.003) set of greenhouse trials (Figure 3A,B). Not surprisingly, time after infestation also had a highly significant effect on aphid population counts in both the first ($F_{6,60} = 860.5$, P<0.001) and the second ($F_{4,40} = 564.7$, P<0.001) set of greenhouse trials. Block effects did not significantly impact aphid population growth in the first set of trials ($F_{2,10} = 0.53$, P = 0.603), but did have a small significant effect on aphid population growth in the second set of trials ($F_{2,10} = 5.95$, P = 0.020). In the first set of greenhouse studies, post-hoc comparisons using Dunnett's test revealed that mean
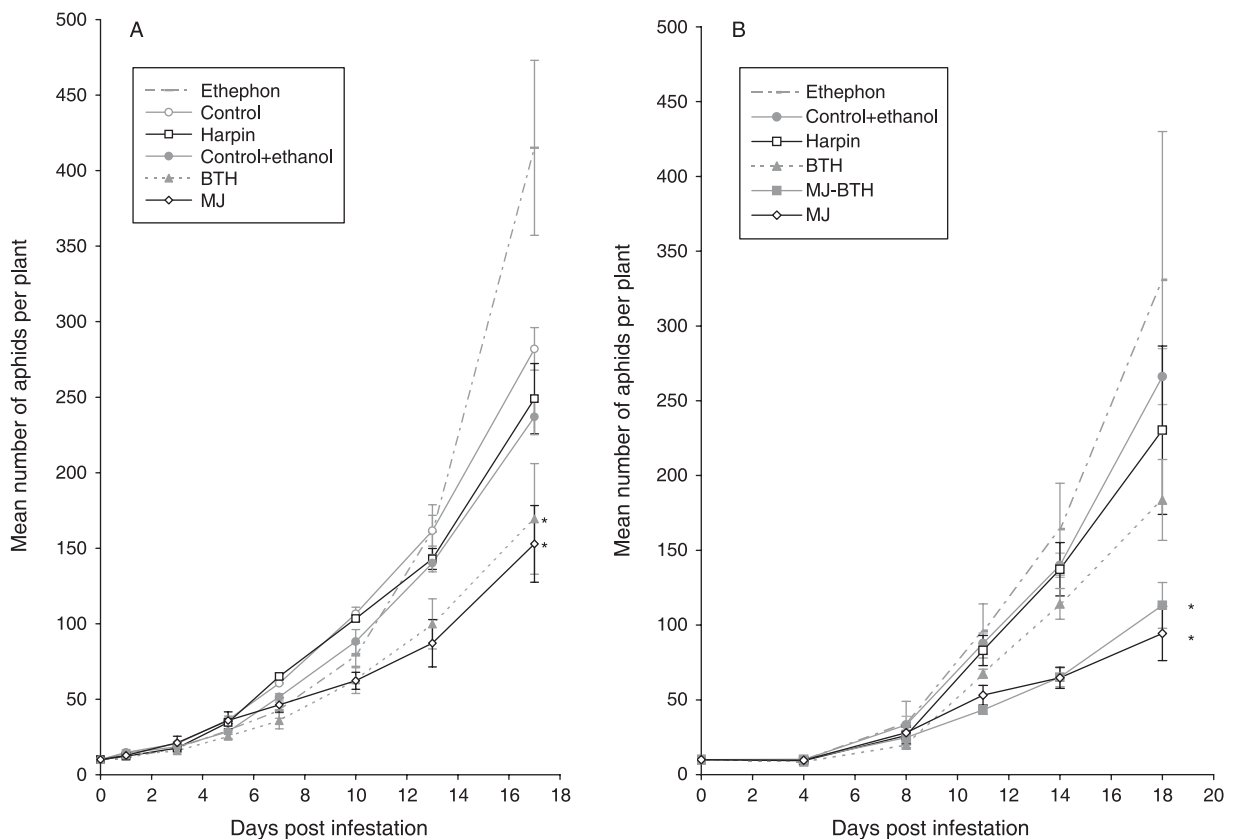


**Figure 3** Graphs showing population growth of *Myzus persicae* on elicitor-treated plants. (A) November 2003 trial and (B) January 2004 trial. Population means based on aphid counts on nine plants per treatment at each time point. Bars show SEMs, based on n = 3 (three separate cages per treatment, with three plants per cage). Asterisks indicate treatments where aphid populations are significantly lower (P<0.05) than on control-treated plants (based on analysis of square-root transformed data).

**Table 2** Per capita rates of growth for *Myzus persicae* populations on elicitor-treated plants and corresponding population doubling times

| Treatment | November 2003 trial | | January 2004 trial | |
|---|---|---|---|---|
| | r coefficient[1] | Population doubling time (days) | r coefficient[2] | Population doubling time (days) |
| BTH | 0.174 | 4.0 | 0.156 | 4.4 |
| Harpin | 0.209 | 3.3 | 0.172 | 4.0 |
| Ethephon | 0.214 | 3.2 | 0.190 | 3.7 |
| MJ | 0.175 | 4.0 | 0.129 | 5.4 |
| Control | 0.215 | 3.2 | – | – |
| Control + ethanol | 0.201 | 3.5 | 0.180 | 3.9 |
| MJ–BTH | – | – | 0.130 | 5.4 |

[1]Estimate of per capita rates of growth. $R^2$ values of fitted exponential curves ranged between 0.95 and 0.99.
[2]Estimate of per capita rates of growth. $R^2$ values of fitted exponential curves ranged between 0.88 and 0.94.
MJ, methyl jasmonate; BTH, benzothiadiazole.

aphid population counts averaged across sampling points were significantly lower on plants treated with BTH (P = 0.017) or MJ (P = 0.026) than on plants receiving the water-only control (Figure 3A). Mean aphid population counts were not significantly lower on plants receiving harpin (P = 0.611), ethephon (P = 0.785), or the water and ethanol control (P = 0.335) than counts on plants receiving the water-only control. Results were similar in the second set of greenhouse studies. Mean aphid population counts averaged across sampling points were significantly lower on plants treated with MJ (P = 0.005) or MJ–BTH (P = 0.005) than on plants treated with the water and ethanol control (Figure 3B). Mean aphid population counts were also lower, but not significantly different, on plants treated with BTH (P = 0.126) or harpin (P = 0.541), relative to plants treated with the water–ethanol control. Mean aphid counts were higher on plants treated with ethephon than on plants treated with the water and ethanol control.

Examination of per capita rates of growth 'r' and corresponding projections of population doubling times (Table 2) revealed that treatment of plants with the most effective elicitors would be expected to extend the time required for aphid populations to double by 25–30% relative to aphids on control-treated plants. The most effective elicitors at slowing aphid population growth in the first set of greenhouse trials were BTH and MJ, while in the second set of trials, the most effective treatments were MJ and the MJ–BTH mixture.

### Effects on aphid fecundity

Aphid fecundity data were square-root transformed prior to analysis. Total lifetime aphid fecundity was significantly affected by elicitor treatment ($F_{2,40} = 4.05$, P = 0.025), but not by block effects ($F_{2,40} = 0.12$, P = 0.886) (Figure 4A). Founder aphids on tomato plants treated with BTH or MJ produced fewer offspring nymphs over the course of their lifetime than founder aphids on plants treated with a water–ethanol control solution. Mean lifetime fecundity was significantly lower on BTH-treated plants (7.1 ± 1.6 nymphs) than on control-treated plants (19.2 ± 3.3 nymphs). Mean lifetime fecundity was also lower on MJ-treated plants (13.9 ± 3.0 nymphs) than on control plants, but this difference was not significant. Mean time to onset of reproduction was not significantly affected by elicitor treatment ($F_{2,34} = 0.92$, P = 0.410) or block effects ($F_{2,34} = 0.36$, P = 0.698) and was similar for founder aphids on control-treated (11.5 ± 0.6 days), BTH-treated (12.7 ± 0.8 days), and MJ-treated plants (12.1 ± 0.6 days) (Figure 4B). Mean number of reproductive days (defined as days on which one or more nymphs were produced) was not significantly affected by elicitor treatment ($F_{2,40} = 2.31$, P = 0.113) or block effects ($F_{2,40} = 0.14$, P = 0.873), although mean number of reproductive days for founder aphids on BTH-treated plants (5.7 ± 1.2 days) was only about half that for aphids on control-treated plants (10.7 ± 1.7 days), and was also less than for aphids on MJ-treated plants (9.0 ± 1.6 days) (Figure 4C). Mean daily production of nymphs (defined as the total number of offspring nymphs divided by the number of days from onset of reproduction to death), was not significantly affected by elicitor treatment ($F_{2,40} = 2.75$, P = 0.076) or block effects ($F_{2,40} = 1.12$, P = 0.335). However, founder aphids on BTH-treated plants produced only about half as many offspring per day (0.6 ± 0.1 nymphs) as aphids on control-treated plants (1.1 ± 0.1 nymphs), and also produced fewer offspring per day than aphids on MJ-treated plants (0.8 ± 0.1 nymphs) (Figure 4D). Mean time to death of founder aphids was not significantly affected by elicitor treatment ($F_{2,40} = 1.00$, P = 0.377) or block effects ($F_{2,40} = 0.14$, P = 0.869), although founder aphids on BTH-treated plants died sooner (23.7 ± 2.5 days) than aphids
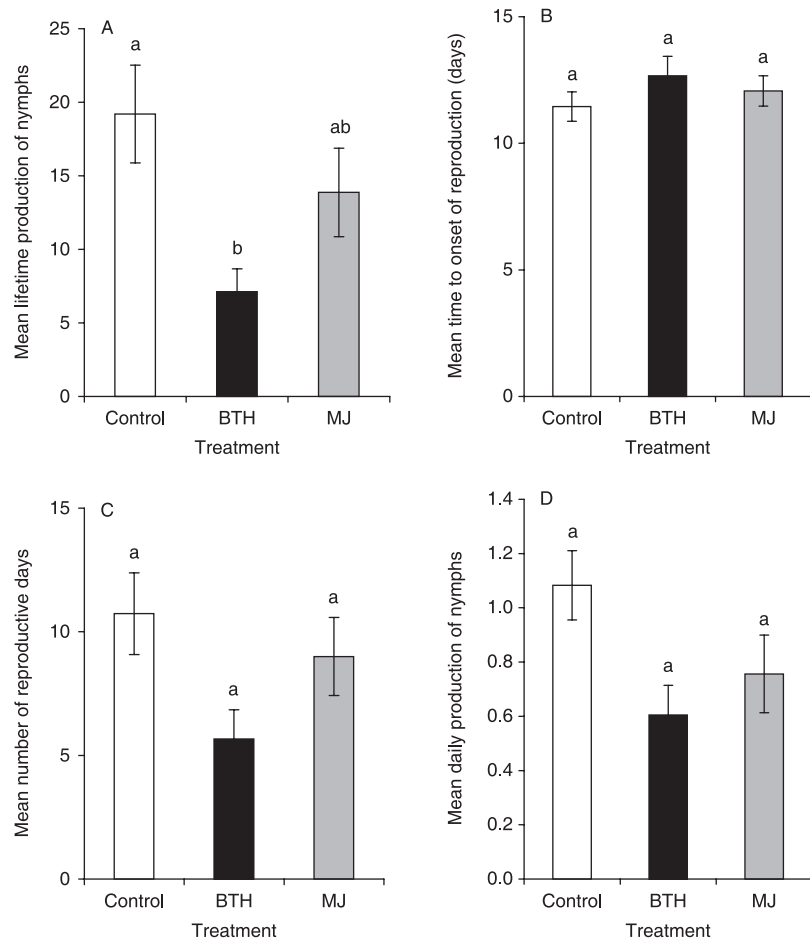
**Figure 4** Histograms showing fecundity data for *Myzus persicae* on plants treated with different elicitors. (A) Mean lifetime production of nymphs, (B) mean time to onset of nymph production, (C) mean number of days when one or more offspring nymphs were produced, and (D) mean number of offspring nymphs produced per day. Means based on 15 founder aphids (one aphid per plant) per treatment. Bars show SEMs. Bars with different letters are significantly different by Tukey's test (P<0.05) (based on analysis of square-root transformed data).

on control-treated (27.7 ± 3.0 days) or MJ-treated (29.9 ± 3.0 days) plants.

Age-specific life tables (Tables 3, 4, and 5) constructed from the aphid fecundity data enabled calculation of the net reproductive rates ($R_0$) of aphid populations on plants treated with different elicitors. Net reproductive rate is defined as the number of breeding offspring that will be produced by each breeding individual in a population. Estimates of $R_0$ for aphids on BTH-treated plants ($R_0$ = 7.1) were substantially lower than estimates on either MJ-treated ($R_0$ = 13.9) or control-treated ($R_0$ = 18.3) plants, providing independent validation of the results of the aphid

**Table 3** Age-specific life table for *Myzus persicae* on control-treated plants

| Age interval (days) | Alive at start $n_x$ | Died during $d_x$ | Proportion surviving $l_x$ | Mortality rate $q_x$ | Number offspring $F_x$ | Fecundity $m_x$ | $l_x m_x$ |
|---|---|---|---|---|---|---|---|
| 0−5 | 15 | 0 | 1.00 | 0.00 | 0 | 0.0 | 0.0 |
| 5−10 | 15 | 1 | 1.00 | 0.07 | 15 | 1.0 | 1.0 |
| 10−15 | 14 | 3 | 0.93 | 0.21 | 72 | 5.1 | 4.8 |
| 15−20 | 11 | 1 | 0.73 | 0.09 | 57 | 5.2 | 3.8 |
| 20−25 | 10 | 2 | 0.67 | 0.20 | 45 | 4.5 | 3.0 |
| 25−30 | 8 | 0 | 0.53 | 0.00 | 44 | 5.5 | 2.9 |
| 30−35 | 8 | 4 | 0.53 | 0.50 | 26 | 3.3 | 1.7 |
| 35−40 | 4 | 3 | 0.27 | 0.75 | 12 | 3.0 | 0.8 |
| 40−45 | 1 | 1 | 0.07 | 1.00 | 4 | 4.0 | 0.3 |

$R_0$ = 18.3.

**Table 4**  Age-specific life table for *Myzus persicae* on benzothiadiazole-treated plants

| Age interval (days) | Alive at start $n_x$ | Died during $d_x$ | Proportion surviving $l_x$ | Mortality rate $q_x$ | Number offspring $F_x$ | Fecundity $m_x$ | $l_x m_x$ |
|---|---|---|---|---|---|---|---|
| 0–5 | 15 | 0 | 1.00 | 0.00 | 0 | 0.0 | 0.0 |
| 5–10 | 15 | 1 | 1.00 | 0.07 | 7 | 0.5 | 0.5 |
| 10–15 | 14 | 3 | 0.93 | 0.21 | 37 | 2.6 | 2.5 |
| 15–20 | 11 | 5 | 0.73 | 0.45 | 26 | 2.4 | 1.7 |
| 20–25 | 6 | 0 | 0.40 | 0.00 | 15 | 2.5 | 1.0 |
| 25–30 | 6 | 1 | 0.40 | 0.17 | 12 | 2.0 | 0.8 |
| 30–35 | 5 | 3 | 0.33 | 0.60 | 6 | 1.2 | 0.4 |
| 35–40 | 2 | 2 | 0.13 | 1.00 | 4 | 2.0 | 0.3 |

$R_0 = 7.1$.

population growth studies and confirming that aphid populations on BTH-treated plants would be expected to grow significantly more slowly than on untreated or control plants.

## Discussion

Several of the elicitors evaluated in this study caused phytotoxicity in treated plants. All doses of ethephon produced rapid and pronounced changes in plant growth form. In the case of the intermediate and high doses of ethephon, these changes in plant growth form were persistent, while for the lowest ethephon concentration, plants partially recovered and went on to flower and produce fruit. These observations of altered plant growth habit were unexpected not only because ethephon is the active ingredient in certain commercial fruit ripening products recommended for use on tomatoes, but also because the concentrations of 1–10 mM used in these studies were not reported to cause abnormal effects in studies of other solanaceous plants (Hong et al., 2000; Lee et al., 2001). MJ treatments also caused some phytotoxicity in the form of necrotic leaf spots, the extent of which appeared to be proportional to MJ concentration, similar to reports for 10 mM JA

treatments in previous studies (Thaler et al., 1996). Foliage of plants sprayed with MJ solutions changed to a noticeably lighter shade of green than control-treated plants during the first 10–14 days after treatment, but their growth form was unaffected and they went on to flower and produce fruit normally. No apparent phytotoxic effects were observed with either of the commercial products BTH or harpin.

Ethephon and MJ treatments also impacted plant growth. Plants' heights measured 20 days after treatment showed that ethephon- and MJ-treated plants were consistently smaller than control plants or those treated with BTH or harpin. Significant dose effects on plant growth were detected for ethephon-treated plants, where the small size of plants treated with intermediate or high concentrations of ethephon appeared to be due to permanent stunting of growth. Plants receiving MJ treatments or the lowest ethephon treatment were only slightly smaller than control plants and appeared to have suffered only mild inhibition of growth and development. Further evidence that the reduced size of MJ-treated plants was due to inhibition and slowing of development, was evidenced by the flowering data, which showed that although total flower number (open flowers and flower buds) on MJ-treated plants was

**Table 5**  Age-specific life table for *Myzus persicae* on methyl jasmonate-treated plants

| Age interval (days) | Alive at start $n_x$ | Died during $d_x$ | Proportion surviving $l_x$ | Mortality rate $q_x$ | Number offspring $F_x$ | Fecundity $m_x$ | $l_x m_x$ |
|---|---|---|---|---|---|---|---|
| 0–5 | 15 | 0 | 1.00 | 0.00 | 0 | 0.0 | 0.0 |
| 5–10 | 15 | 1 | 1.00 | 0.07 | 6 | 0.4 | 0.4 |
| 10–15 | 14 | 2 | 0.93 | 0.14 | 49 | 3.5 | 3.3 |
| 15–20 | 12 | 1 | 0.80 | 0.08 | 40 | 3.3 | 2.7 |
| 20–25 | 11 | 1 | 0.73 | 0.09 | 37 | 3.4 | 2.5 |
| 25–30 | 10 | 2 | 0.67 | 0.20 | 24 | 2.4 | 1.6 |
| 30–35 | 8 | 3 | 0.53 | 0.38 | 16 | 2.0 | 1.1 |
| 35–40 | 5 | 2 | 0.33 | 0.40 | 25 | 5.0 | 1.7 |

$R_0 = 13.9$.

only slightly lower than seen on control-treated plants, the number of open flowers was significantly lower on MJ-treated plants. This observation that MJ-treated plants have a lower proportion of open flowers at 20 DPT, together with their smaller size, indicate that MJ applications delay normal plant development, at least in the first few weeks following MJ applications, causing plants to appear to be of a younger developmental age than control plants treated at the same time. In longer-term studies, our observations suggested that MJ-treated plants were able to recover, and by 42 days after treatment, were of similar sizes to control plants.

These findings of negative impacts of MJ applications on the development of tomato plants are consistent with previous reports. Significant delays in fruit-set on tomato plants treated with 1 mM or 10 mM JA, and significant lengthening of fruit ripening time on plants receiving 10 mM JA treatments, have been reported (Redman et al., 2001). Also observed were significant negative impacts of JA-treatments on plant reproduction, causing reductions in fruit number, reductions in seeds per unit fruit weight, and lower seed production per plant. Thaler (1999) previously reported no negative impacts on tomato production associated with JA treatments, although some results suggested that fewer fruits were produced on JA-treated plants. Neither of these studies detected significant differences in plant biomass between JA- and control-treated plants at the end of the growing season (Thaler, 1999; Redman et al., 2001). Reductions in flower numbers but not in number of flower buds were observed on JA-treated plants (Thaler, 1999), but it seems likely that the lower number of flowers on JA-treated plants was simply the result of plants being developmentally delayed relative to control plants, as was documented in our studies.

Elevated concentrations of defense-related proteins such as PPO, POD, lipoxygenase, and protease inhibitors have frequently been documented in tomato leaf tissue in response to wounding or feeding by arthropod herbivores (Stout et al., 1996; Fidantsef et al., 1999). We used activity levels of PPO and POD in leaf tissue from elicitor-treated plants as indicators of whether elicitors had induced plant defensive responses. All concentrations of MJ caused significant increases of approximately threefold in leaf PPO activities relative to control-treated plants, which is consistent with previous studies (Thaler et al., 1996; Constabel & Ryan, 1998). None of the other elicitors induced changes in leaf PPO levels relative to levels observed in leaves from control plants. The situation was more variable in the case of POD. Only doses of ethephon above 5 mM or MJ above 7.5 mM caused statistically significant elevations of leaf POD levels above those seen in control-treated plants. These findings concerning the effect of MJ on POD activities broadly agreed with a previous study which documented elevated

leaf POD levels only in response to JA treatments approaching 10 mM (Thaler et al., 1996).

Aphid populations on plants treated with BTH or MJ grew significantly more slowly than did aphid populations on plants treated with ethephon, harpin, or control solutions. Numerous previous studies have documented reduced performance of chewing herbivores on tomato following activation of the JA-dependent wounding pathway associated with induction of defensive proteins such as PPO and protease inhibitors (Thaler et al., 1996, 1999; Stout et al., 1998a; Cipollini & Redman, 1999; Bostock et al., 2001). However, much less is known about how elicitor-induced responses affect piercing-sucking insect herbivores (Walling, 2000). Our results are in agreement with recent studies demonstrating reduced rates of aphid population growth on BTH- and MJ-treated tomato plants (Cooper et al., 2004; Cooper & Goggin, 2005). Previous studies have questioned whether plant-induced responses to SA (Bi et al., 1997) or BTH (Inbar et al., 2001) provide protection from insect herbivores, but it is difficult to reconcile these conclusions with our findings for several reasons. First, these previous studies were performed on cotton rather than tomato plants. Second, Bi et al. (1997) used SA as an inducer of SAR, and more recent studies using microarray analysis have revealed subtle but important differences in plant transcriptional responses to elicitation by SA and BTH (Heidel & Baldwin, 2004). In agreement with earlier findings on chewing herbivores (Thaler et al., 1999), Inbar et al. (2001) confirmed that plant defenses against *Helicoverpa armigera* were compromised by BTH treatments. However, Inbar (2001) also concluded that BTH-induced SAR had negligible effects against the whitefly *Bemisia tabaci*, although this conclusion was based solely on studies of oviposition preference and did not give weight to the finding of reduced whitefly oviposition on older leaves of BTH-treated plants. In our *M. persicae* population growth studies, we found no evidence of movement of aphids off of BTH-treated plants, indicating that reductions in population growth were due to antibiotic rather than antixenotic factors. As such, oviposition preference may not be the most reliable indicator of whether whiteflies are adversely affected by BTH-induced SAR.

We hypothesized that the reduced rates of *M. persicae* population growth we observed in our studies were either the result of increases in the length of time taken for aphids to reach reproductive age, or were due to reductions in aphid fecundity. Subsequent fecundity studies demonstrated that aphids on BTH-treated plants exhibited significantly lower fecundity than those on control-treated plants, although time taken to reach reproductive age and time to death (data not shown) were not significantly different from aphids on control-treated plants. Reduction in total

fecundity of aphids on BTH-treated plants appeared to be the result of a reduction in the number of days when offspring nymphs were produced combined with the production of fewer offspring nymphs on these productive days. Because treatment of tomato plants with BTH does not induce protease inhibitors in leaves (Fidantsef et al., 1999), and our results demonstrated that total PPO activity was not increased in response to BTH treatments, other mechanisms must be responsible for reductions in aphid fecundity on BTH-treated plants.

The reason for reduced aphid population growth on MJ-treated plants is less clear. Although aphid fecundity was reduced, these reductions were not statistically significant and so other factors must be involved in reducing population growth on MJ-treated plants. Cooper & Goggin (2005) found that offspring of potato aphids on JA-treated tomato plants exhibited significant reductions in survival rates in both the F1 and the F2 generations, and it is possible that similar effects may have contributed to the reduced rate of aphid population growth we observed on MJ-treated plants. At least two serine proteinase inhibitors, tomato proteinase inhibitor I and tomato proteinase inhibitor II (Broadway & Duffey, 1986), are induced in tomatoes following MJ treatments (Farmer & Ryan, 1990), and might be partly responsible for reductions in aphid fecundity and growth. Serine proteinase inhibitors have consistently been shown to reduce larval performance of lepidopteran pests (Broadway & Duffey, 1986; Broadway et al., 1986; Johnson et al., 1989; Ryan, 1990) but their effects on piercing–sucking pests are more variable. Some studies have documented adverse effects of serine proteinase inhibitors on aphid survival and reproduction (Rahbé & Febvay, 1993; Tran et al., 1997; Ceci et al., 2003; Rahbé et al., 2003; Azzouz et al., 2005) while others have documented limited or variable effects (Rahbé et al., 1995; Casaretto & Corcuera, 1998; Voelckel et al., 2004; Hesler et al., 2005). These studies suggest the possibility that proteinase inhibitors induced in tomato following MJ treatments may have negative impacts on population growth of green peach aphids. Previous studies have documented negative interactions between the JA-dependent wound response pathway and the SA-dependent signaling pathways (Felton et al., 1999; Bostock et al., 2001; Thaler et al., 2002; Traw & Bergelson, 2003). This antagonism leads to reduced induction of herbivore defenses, such as proteinase inhibitors and PPO, resulting in compromised resistance to feeding by chewing herbivores such as noctuid caterpillars (Stout et al., 1999; Thaler et al., 1999, 2002; Bostock et al., 2001). If proteinase inhibitors were involved in limiting aphid population growth on MJ-treated plants, antagonism between defensive pathways on plants treated with a mixture of BTH–MJ would be expected to reduce proteinase inhibitor expression, and reduce aphid resistance relative to plants treated with MJ alone. This slight reduction in aphid resistance on MJ–BTH-treated plants is exactly what we found, and is consistent with earlier biochemical studies that documented an approximately 25% reduction in proteinase inhibitor II transcripts in leaves from tomato plants treated with a JA–BTH mixture relative to leaves from plants treated with JA alone (Fidantsef et al., 1999). Harpin treatments had no effect on the growth of *M. persicae* populations, indicating that they were not activating the same resistance mechanisms as those triggered by BTH and MJ treatments.

PPO has also been shown to reduce the efficiency of food digestion in lepidopteran larvae (Felton et al., 1989), although little information is available on the effect of PPO on feeding and food utilization by piercing-sucking insects. A potentially more significant defensive role of PPO in tomato is its presence in type VI glandular trichomes, where it functions to catalyze the oxidation of phenolics that are released when glandular trichomes are ruptured by arthropods on the plant surface (Duffey, 1986). This oxidation of phenolics produces sticky secretions that interfere with mouthparts, tangle appendages, or may entrap small arthropods (Duffey, 1986). Recently we showed that the density of type VI glandular trichomes is significantly increased on new leaves of MJ-treated plants (Boughton et al., 2005) and this might conceivably lead to gumming of aphid appendages and hampered movement. Given that aphid performance has been shown to be negatively correlated with trichome density (Goundoudaki et al., 2003), it seems likely that elevated trichome densities may be partly responsible for reducing aphid population growth on MJ-treated plants.

In agreement with previous studies, the results presented here suggest that treatment of plants with elicitors such as MJ and BTH has the potential to reduce population growth rates and performance of herbivorous insect pests on tomato. More studies are needed however, to shed light on changes induced in tomatoes by BTH treatments and exactly how these induced responses reduce aphid fecundity.

## Acknowledgements

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